Recent Studies on a μ-Opioid Receptor Purified from Bovine Striatum[a]

ERIC J. SIMON

Departments of Psychiatry and Pharmacology
New York University Medical Center
550 First Avenue
New York, New York 10016

The existence of opioid receptors in the central nervous system of animals and humans was demonstrated in 1973 simultaneously by our laboratory[1,2] and by others.[3–5] At the time only a single type of receptor was postulated, a concept soon challenged and disproved. Martin and co-workers[6,7] postulated three classes of receptors, μ, κ, and σ. The latter is now not considered to be an opioid receptor and has been replaced by δ, an enkephalin-preferring receptor first demonstrated by Kosterlitz and co-workers[8] in the mouse vas deferens. The enkephalins are therefore thought to be the endogenous ligands of the δ-receptor, dynorphin that of the κ-receptor, and β-endorphin a putative endogenous ligand of the μ-receptor. Within the past year the δ-opioid receptor was cloned independently by two laboratories.[9,10] This was rapidly followed by the cloning of a μ- and a κ-receptor,[11,12] by low stringency screening of cDNA libraries with oligonucleotide probes from the structure of the δ-receptor cDNA. The protein sequences derived from the cDNA's coding for the three types of opioid receptors are shown in FIGURE 1. All of them have structures with seven putative transmembrane domains, typical of G-protein coupled receptors. The figure shows a number of receptors of this class that exhibit high levels of homology with the opioid receptors.

There is considerable pharmacological evidence for the existence of multiple subtypes of each of the three major types of opioid receptors. However, no evidence for subtypes has yet been provided by cloning, that is, all clones of a given type, obtained in a number of laboratories,[13–19] including those cited earlier, have proved to be identical to the cDNA's coding for the μ-, δ-, and κ-opioid receptors originally published. The present paper discusses recent work in our laboratory on a μ-opioid receptor purified to homogeneity from bovine striatal membranes, which appears to be a subtype, different from the μ-receptor which has been cloned in several laboratories. Final proof of this hypothesis awaits the cloning of this protein.

RESULTS AND DISCUSSION

Purification and Microsequencing of a μ-Opioid Binding Protein

The purification to homogeneity of an opioid binding protein (OBP) from bovine striatal membrane was reported by us some years ago.[20] Briefly, the membranes were solubilized with digitonin and the extract was purified in two steps, ligand-affinity chromatography, which provided the major purification (4000- to 5000-fold) fol-

[a]This work is supported by National Institute on Drug Abuse grant DA-00017. Gifts from Hoffmann-LaRoche, Inc. and Pfizer Central Research are gratefully acknowledged.

lowed by lectin-affinity chromatography on wheat-germ agglutinin agarose, for a total purification of 60,000- to 70,000-fold. The purified protein gives a single band on SDS-polyacrylamide gel electrophoresis corresponding to a molecular mass of 65 kDa. It is highly glycosylated and enzymatic deglycosylation yields a protein of 40–43 kDa (Gioannini and Simon, unpublished results). Preliminary evidence suggested that it has the properties of a μ-opioid receptor, though this evidence was not conclusive because the purified protein bound only antagonists with high affinity. High-affinity agonist binding was lost, presumably due to uncoupling of the receptor protein from G-protein (see below for experiments on reconstitution).

The purified protein was found to be blocked at the N-terminus. Fragmentation with cyanogen bromide and sequencing, kindly carried out for us by Dr. C. Strader at Merck & Co., provided two peptides, one 20 amino acids long (peptide I) and the other 13 amino acids in length (peptide II). More recently, Dr. W. Burgess at the American Red Cross obtained four more short peptides for us by fragmentation with lysine-protease and microsequencing. It is of interest that none of these six peptides are found in the published amino acid sequence of the cloned μ-opioid receptor. Attempts to clone OBP, using PCR and direct screening of cDNA and genomic libraries, with oligonucleotides made from peptides I and II, have not yet succeeded. This is presumably because of the relatively short peptides and their high level of degeneracy. The following sections deal with studies using antibodies (Abs) made against portions of peptide I and II and with the reconstitution of purified OBP with G-proteins in liposomes.

Antisera against Peptides Derived from Opioid Binding Protein

Our laboratory, in collaboration with Dr. Lawrence Taylor in Dr. Huda Akil's laboratory, University of Michigan (Ann Arbor, MI), produced six antisera against three sequences in peptides I and II.[21] TABLE 1 lists the sequences and the Abs made against them. All of these Abs recognized the purified OBP, from which the peptides are derived. They immunoprecipitate the bulk of radioiodine-labeled OBP and give an immunoblot at the correct molecular mass of 65 kDa. Most of our subsequent studies were done with antiserum Ab165, made against the N-terminal 12 amino acids of peptide I, the most immunoreactive serum against the OBP protein. In studies of a number of bovine brain regions, positive immunoblot signals corresponding to a molecular mass of 65 kDa were obtained with regions known to be rich in μ-receptors, whereas no signal was obtained with regions devoid of opioid receptors, such as white matter. Similarly, the cell line, SK-N-SH, high in μ-opioid receptors, gave a band in immunoblots, whereas HeLa and C-6 cells, devoid of opioid receptors, do not. Interestingly, NG-108-15 cells that express only δ-receptors give a band at a slightly lower molecular mass of approximately 60 kDa with Ab165. This may suggest cross-reaction with δ-receptors or the presence of silent and slightly different μ-receptors. Experiments are in progress to determine which of these alternatives is correct.

More recently, Dr. Hiller in our laboratory has carried out immunocytochemical studies of the distribution of Ab165-reactive material in the rat central nervous system.[22] It parallels very closely the distribution of μ-opioid receptor binding, determined by autoradiography by ourselves[23] and by others.[24,25] FIGURE 2 shows an example of these studies, namely, the spinal cord distribution of immunoreactivity to Ab165.

	1	#		#	# 50
MUOR1	.......MDS	STGPGNTSDC	SDPLAQASCS	PAPGSWLNLS	HVDGNQSDPC
DOR1	....	....	MELVPSARAE	LQSSPLVNLS	.....DAFPS
KOR1	....	......MESP	IQIFRGDPGP	TCSPSACLLP	NSSSWFPNWA
SOMR1	....	....	..MFPNAPPP	LPHSSPSSSP	GGCGEGVCSR
FPEP	....	....	....	....	....
OBPR	....	......MASP	AGNLS.AWPG	WGWPP..PAA	LRNLTSSPAP
NEUROKR	MASVPRGENW	TDGTVEVGTH	TGNLSSALGV	TEWLALQAGN	FSSALGLPAT
B2AR	....	....	....	....	.......MEP
	51# #				100
MUOR1	GLNRTGLGGN	DSLCPQTG..	...SPSMVTA	ITIMALYSIV	CVVGLFGNFL
DOR1	AFPSAGANAS	GSPGARSA..	...S.SLALA	IAITALYSAV	CAVGLLGNVL
KOR1	ESDSNGSVGS	EDQQLESA..	...HISPAIP	VIITAVYSVV	FVVGLVGNSL
SOMR1	GPGSGAADGM	EEPGRNSSQN	GTLSEGQGSA	ILISFIYSVV	CLVGLCGNSM
FPEP	....METNSS	LPTNISGGTP	AVSAGYLFLD	IITYLVFAVT	FVLGVLGNGL
OBPR	TASPSPAPSW	TPSPRPGPAH	PFLQPPWAVA	LWSLA.YGAV	VAVAVLGNLV
NEUROKR	TQAPSQV...	....RANLTN	QFVQPSWRIA	LWSLA.YGLV	VAVAVFGNLI
B2AR	HGNDSDFLLA	PNGSRAPGHD	ITQERDEAWV	VGMAILMSVI	VLAIVFGNVL
	101				150
MUOR1	VMYVIVRYTK	MKTATNIYIF	NLALADALAT	STLP.FQSVN	YLMGTWPFGT
DOR1	VMFGIVRYTK	LKTATNIYIF	NLALADALAT	STLP.FQSAK	YLMETWPFGE
KOR1	VMFVIIRYTK	MKTATNIYIF	NLALADALVT	TTMP.FQSAV	YLMNSWPFGD
SOMR1	VIYVILRYAK	MKTATNIYIL	NLAIADELLM	LSVP.FLVTS	TLLRHWPFGA
FPEP	VIWVAG.FRM	THTVTTISYL	NLAVADFCFT	STLPFFMVRK	AMGGHWPFGW
OBPR	VIWIVLAHKR	MRTVTNSFLV	NLAFADAAMA	ALNALVNFIY	ALHGEWYFGA
NEUROKR	VIWIILAHKR	MRTVTNYFLV	NLAFSDASVA	AFNTLINFIY	GLHSEWYFGA
B2AR	VITAIAKFER	LQTVTNYFIT	SLACADLVMG	LAVVPFGASH	ILMKMWNFGN
	151				200
MUOR1	ILCKIVISID	YYNMFTSIFT	LCTMSVDRYI	AVCHPVKALD	FRTPRNAKIV
DOR1	LLCKAVLSID	YYNMFTSIFT	LTMMSVDRYI	AVCHPVKALD	FRTPAKAKLI
KOR1	VLCKIVISID	YYNMFTSIFT	LTMMSVDRYI	AVCHPVKALD	FRTPLKAKII
SOMR1	LLCRLVLSVD	AVNMFTSIYC	LTVLSVDRYV	AVEHPIKAAR	YRRPTVAKVV
FPEP	FLCKFVFTIV	DINLFGSVFL	IALIALDRCV	CVLHPVWTQN	HRTVSLAKKV
OBPR	NYCRFQNFFP	ITAVFASIYS	MTAIAVDRYM	AIIDPLKPR.	.LSATATRIV
NEUROKR	NYCRFQNFFP	ITAVFASIYS	MTAIAVDRYM	AIIDPLKPR.	.LSATATKIV
B2AR	FWCEFWTSID	VLCVTASIET	LCVIAVDRYV	AITSPFKYQS	LLTKNKARVV
	201				250
MUOR1	NVCNWILSSA	IGLPVMFMAT	TKYRQ..GSI	DCTL.TFS..	..HPTW.YWE
DOR1	NICIWVLASG	VGVPIMVMAV	TQPRD..GAV	VCML.QFP..	..SPSW.YWD
KOR1	NICIWLLASS	VGISAIVLGG	TKVREDVDVI	ECSL.QFP..	..DDEYSWWD
SOMR1	NLGVWVLSLL	VILPIVVFSR	TAANSD.GTV	ACNM.LMP..	..EPAQRWLV
FPEP	IIGPWVMALL	LTLPVIIRVT	T.VPGKTGTV	ACTF.NFSPW	TNDPKERIKV
OBPR	IGSIWILAFL	LAFPQCLYSK	IKVMP.....	GRTL.CYVQW	PEGSRQHFTY
NEUROKR	IGSIWILAFL	LAFPQCLYSK	IKVMP.....	GRTL.CYVQW	PEGPKQHFTY
B2AR	ILMVWIVSGL	TSFLPIQMHW	YRATHKQAID	CYAKETCCDF	FTNQAYAIAS

FIGURE 1. Amino acid sequences of the cloned μ-, δ-, and κ-opioid receptors and homologous G-protein coupled receptors. Boldface type and shading: putative transmembrane domains. *, putative sites for phosphorylation by protein kinase A. #, putative sites for N-linked glycosylation. (Adapted from Wang *et al.*,[13] with permission.)

Reconstitution of High-Affinity Binding and GTPase Stimulation

Our preliminary pharmacological evidence as well as the results of the studies with the antibodies directed against peptides from purified OBP suggested that OBP was a μ-binding protein. However, these results were not conclusive, and real proof had to come from pharmacological studies of the reconstituted purified OBP, described in this section.

```
          251                                                       300
  MUOR1   NLLKICV... ..FIFAFIMP VLIITVCYGL MILRLKSVRM LSGS....KE
   DOR1   TVTKICV... ..FLFAFVVP ILIITVCYGL MLLRLRSVRL LSGS....KE
   KOR1   LFMKICV... ..FVFAFVIP VLIIIVCYTL MILRLKSVRL LSGS....RE
  SOMR1   GFV.LYT... ..FLMGFLLP VGAICLCYVL IIAKMRMVPS RPAG.....S
   FPEP   AVAMLTVRGI IRFIIGFSAP MSIVAVSYGL IATKIHKQGL IKSS......
   OBPR   HMIVI..... ...VLVYCFP LLIMGITYTI VGITLWGGEI PGDTCDKYQE
NEUROKR   HIIVI..... ...ILVYCFP LLIMGVTYTI VGITLWGGEI PGDTCDKYHE
   B2AR   SIVSFYVP.. .LVVMVFVYS RVFQVAKRQL QKIDKSEGRF HAQNLSQVEQ

          301                 *                                 350
  MUOR1   KDRN..... .......LRR ITRMVLVVVA VFIVCWTPIH IYVIIKALIT
   DOR1   KDRS..... .......LRR ITRMVLVVVG AFVVCWAPIH IFVIVWTLVD
   KOR1   KDRN..... .......LRR ITKLVLVVVA VFIICWTPIH IFILVEALGS
  SOMR1   TQRS..... .......ERK ITLMVMMVVM VFVICWMPFY VVQLVNVFAE
   FPEP   ..........  .........R PLRVLSFVAA AFFLCWSPYQ VVALIATVRI
   OBPR   QLKA..... .......KRK VVKMMIIVVV TFAICWLPYH IYFILTAIYQ
NEUROKR   QLKA..... .......KRK VVKMMIIVVV TFAICWLPYH VYFILTAIYQ
   B2AR   DGRSGHGLRS SSKFCLKEHK ALKTLGIIMG TFTLCWLPFF IVNIVHVIRA

          351                                                       400
  MUOR1   I.PETTF... QTVSWHFCIA LGYTNSCLNP VLYAFLDENF KRCF.REF..
   DOR1   INRRDPL... VVAALHLCIA LGYANSSLNP VLYAFLDENF KRCF.RQL..
   KOR1   TSHSTA.... ALSSYYFCIA LGYTNSSLNP VLYAFLDENF KRCF.RDF..
  SOMR1   QDDATVS... Q.....LSVI LGYANSCANP ILYGFLSDNF KRSFQRIL..
   FPEP   RELLQGMYKE IGIAVDVTSA LAFFNSCLNP MLYVFMGQDF RERLIHAL..
   OBPR   QLNRWKYIQQ VYLASFW... LAMSSTMYNP IIYCCLNKRF RAGFKRAFRW
NEUROKR   QLNRWKYIQQ VYLASFW... LAMSSTMYNP IIYCCLNKRF RAGFKRAFRW
   B2AR   NLIPKEV... ....YILLNW LGYVNSAFNP LIYC.RSPDF RIAFQELLCL

          401                                                       450
  MUOR1   .CIPTSSTIE QQNSTRVRQN TREHPSTANT VDRTNHQLEN LEARTAPLP.
   DOR1   .CRTPCGRQE PGSLRRPRQA TTRERVTACT PS......DG PGGGAAA...
   KOR1   .CFPIKMRME RQSTNRVR.N TVQDPASMRD VGGMNKPV.. ..........
  SOMR1   .CL...SWMD NAAEEPVDYY ATALKSRAYS VEDFQPENLE SGGVFRNGTC
   FPEP   .PASLERALT EDSTQTSDTA TNSTLPSAEV ALQAK..... ..........
   OBPR   CPFIHVSSYD ELELKATRLH PMRQ.SSLYT VTRMESMSVV FDSNDGDSAR
NEUROKR   CPFIQVSSYD ELELKTTRFH PTRQ.SSLYT VSRMESVTVL FDPNDGDPTK
   B2AR   RRSSSKTYGN GYSSNSNGRT DYTGEQSAYQ LGQEKENELL CEEAPGMEGF
```

FIGURE 1. (*continued*)

Our initial studies of OBP reconstitution[26] were done with a CHAPS extract of bovine striatal membranes as the source of phospholipids and G-proteins. This extract was prepared in the absence of sodium, which we have shown to solubilize very little opioid binding activity. Heating the extract at 37 °C for 30 min removed any residual traces of opioid binding activity. Lipid vesicles were formed by adding purified OBP to the CHAPS extract, precipitating the mixture with polyethyleneglycol-6000 (PEG), and resuspending the pellet, resulting from centrifugation at 12,000 × *g*,

TABLE 1. Antipeptide Antibodies Generated against Amino Acid Sequences Derived from Opioid Binding Protein

	Amino Acid Sequence	Antibody
Peptide I-1-12	IRNLRQDRSKYY[X]	165, 166, 6639
Peptide I-14-20	NFFYKRL	163
Peptide II-1-9	Y*SNNVLFVSH[XFND]	161, 162

NOTE: *, Y-tyrosine added to permit iodination of peptide. X indicates unknown amino acids. Bracketed residues were not used for antibody production. (Gioannini *et al.*[21] Reprinted with permission from *Molecular Pharmacology.*)

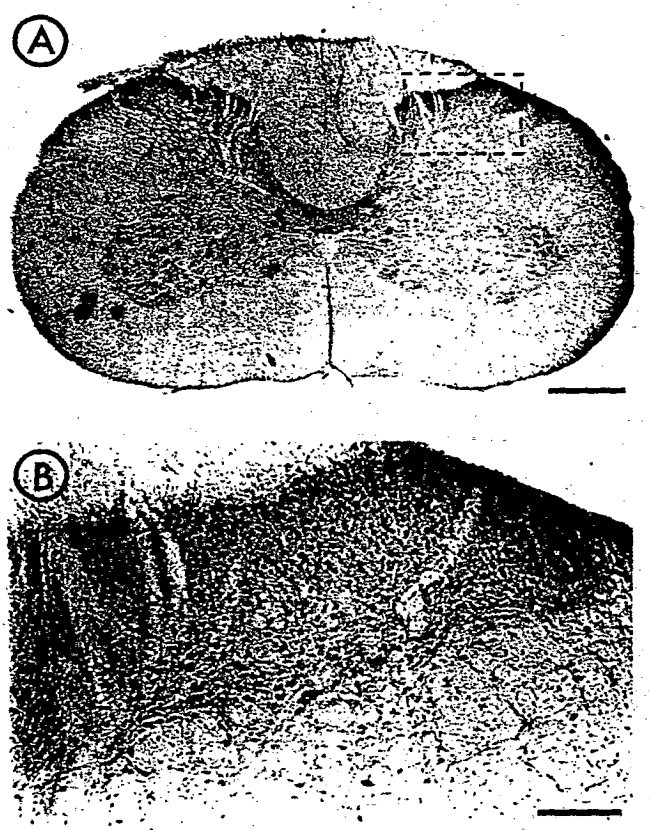

FIGURE 2. Distribution of immunoreactivity against Ab165 in the rat lumbar spinal cord. (A) High levels of immunoreactivity are seen in lamina I and lamina II (substantia gelatinosa) of the dorsal horn. Higher magnification of the (boxed area of panel A) dorsal horn, shown in panel B, reveals that laminae III and IV also contain immunopositive cell bodies. An edge artifact is apparent in the upper right corner of this panel. It is also notable that the median aspect of lamina V (see panel A) contains a substantial level of immunoreactivity. In the ventral horns, large individual immunopositive cell bodies can be seen in laminae VII, VIII, and IX. Bars, A = 100 μm, B = 2 μm. (Hiller *et al.*[22] Reproduced, with permission, from *Neuroscience.*)

in Tris buffer containing magnesium. FIGURE 3 shows that the selective μ-agonist DAGO binds saturably and with high affinity to the reconstituted OBP. The figure also shows that the binding is very selective for μ-ligands. The binding was found to be completely dependent on and proportional to the amount of OBP added to the liposomes, stereospecific and sensitive to inhibition by GTPγS. The affinity of DAGO for reconstituted OBP was 1.5 nM, identical to that seen in the membrane-bound μ-opioid receptor in bovine striatal membranes. Functional coupling was also achieved, as evidenced by the stimulation of low K_m GTPase by μ-agonists, such as DAGO.

More recently we repeated the reconstitution studies with purified G-proteins in collaboration with the laboratory of John Hildebrandt at South Carolina Medical University (Charleston, SC) (manuscript in preparation). Most of the studies were carried out with a highly purified mixture of brain G-proteins, composed of 80% G_{oA}, 7–8% G_{oC}, 7–8% G_{il} and small amounts of G_{oB} and G_{i2}. Some experiments with individual purified G-proteins were also done. The liposomes were formed by mixing purified OBP with phosphatidylcholine (Sigma) and the purified G-proteins. The mixture was precipitated with PEG, centrifuged at 100,000 × *g*, and resuspended in Tris buffer containing magnesium. Functional reconstitution was assessed by measurement of opioid stimulation of low K_m GTPase. Opioid ligands, such as DAGO, morphine and levorphanol, produced up to 100% stimulation of GTPase. The stimulation was reversed by (−)naloxone but not by (+)naloxone. Dextrorphan, the inactive enantiomer of levorphanol, was inactive and so were δ- and κ-agonists.

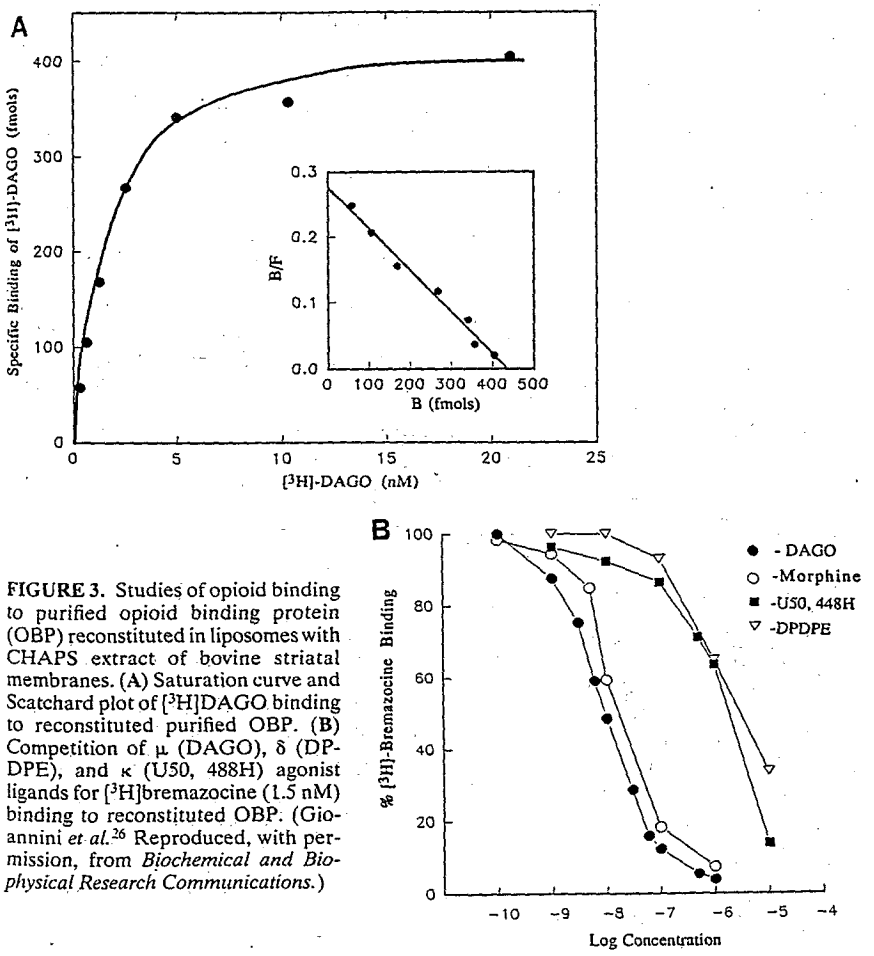

FIGURE 3. Studies of opioid binding to purified opioid binding protein (OBP) reconstituted in liposomes with CHAPS extract of bovine striatal membranes. (**A**) Saturation curve and Scatchard plot of [^{3}H]DAGO binding to reconstituted purified OBP. (**B**) Competition of μ (DAGO), δ (DPDPE), and κ (U50, 488H) agonist ligands for [^{3}H]bremazocine (1.5 nM) binding to reconstituted OBP. (Gioannini *et al.*[26] Reproduced, with permission, from *Biochemical and Biophysical Research Communications.*)

Studies of μ-agonist binding showed that high-affinity binding to reconstituted OBP was obtained both with the G-protein mixture and with individual G-proteins. Differences in efficacy between different G-proteins were not significant. Furthermore, differences observed could represent differences in incorporation of various G-proteins into liposomes, in the level of coupling or in the nature of the coupling. We cannot at present distinguish among these possibilities. It should be noted that the level of binding and the affinity of DAGO binding were lower than those seen when CHAPS extract was the source of G-proteins. This could be due to the absence of factors that favor high levels of incorporation into liposomes and coupling or to some slight denaturation of G-proteins during purification. The affinity of DAGO binding, $K_d = 7$ nM, was high but somewhat lower than the $K_d = 1.5$ nM we observed in the experiments with CHAPS extract.

In summary, our studies with antisera directed against peptides derived from purified OBP, and especially the pharmacological profile obtained with reconstituted OBP, demonstrate clearly that we have purified a μ-receptor. The fact that none of the peptides obtained from the purified protein are identical to sequences in the cloned μ-receptor suggests that it may be a different subtype, the predominant subtype in bovine brain. Our results suggest that this subtype of μ-opioid receptor is also present in rat brain or, alternatively, that sequence homology and/or tertiary structural similarities give rise to cross-reactivity of the rat μ-receptor with our antisera.

REFERENCES

1. SIMON, E. J., J. M. HILLER & I. EDELMAN. 1973. Stereospecific binding of the potent narcotic analgesic ^{3}H-etorphine to rat brain homogenate. Proc. Natl. Acad. Sci. USA **70:** 1947–1949.
2. HILLER, J. M., J. PEARSON & E. J. SIMON. 1973. Distribution of stereospecific binding of the potent narcotic analgesic etorphine in the human brain: Predominance in the limbic system. Res. Commun. Chem. Pathol. Pharmacol. **6:** 1052–1062.
3. PERT, C. B. & S. H. SNYDER. 1973. Opiate receptor: Demonstration in nervous tissue. Science **179:** 1011–1014.
4. KUHAR, M. J., C. B. PERT & S. H. SNYDER. 1973. Regional distribution of opiate receptor binding in monkey and human brain. Nature **245:** 447–451.
5. TERENIUS, L. 1973. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacol. Toxicol. **32:** 317–320.
6. MARTIN, W. R., C. G. EADES, J. A. THOMPSON, R. E. HUPPLER & P. E. GILBERT. 1976. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. **197:** 517–532.
7. GILBERT, P. E. & W. R. MARTIN. 1976. The effects of morphine- and nalorphine-like drugs in the nondependent morphine-dependent and cyclazocine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. **198:** 66–82.
8. LORD, J. A. H., A. A. WATERFIELD, J. HUGHES & H. W. KOSTERLITZ. 1977. Endogenous opioid peptides: Multiple agonists and receptors. Nature **267:** 495–499.
9. EVANS, C. J., D. E. KEITH, JR., H. MORRISON, K. MAGENDZO & R. H. EDWARDS. 1992. Cloning of a delta opioid receptor by functional expression. Science **258:** 1952–1955.
10. KIEFFER, B. L., K. BEFORT, C. GAVERIAUX-RUFF & C. G. HIRTH. 1992. The delta-opioid receptor: Isolation of a cDNA by expression cloning and pharmacological characterization. Proc. Natl. Acad. Sci. USA **89:** 12048–12052.
11. CHEN, Y., A. MESTEK, J. LIU, J. A. HURLEY & L. YU. 1993. Molecular cloning and functional expression of a mu-opioid receptor from rat brain. Mol. Pharmacol. **44:** 8–12.
12. YASUDA, K., K. RAYNOR, H. KONG, C. D. BREDER, J. TAKEDA, T. REISINE & G. I. BELL.

1993. Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. Proc. Natl. Acad. Sci. USA **90:** 6736–6740.

13. WANG, J. B., Y. IMAI, C. M. EPPLER, P. GREGOR, C. E. SPIVAK & G. R. UHL. 1993. Mu opiate receptor: cDNA cloning and expression. Proc. Natl. Acad. Sci. USA **90:** 10230–10234.
14. MINAMI, M., T. TOYA, Y. KATAO, K. MAEKAWA, S. NAKAMURA, T. ONOGI, S. KANEKO & M. SATOH. 1993. Cloning and expression of a cDNA for the rat kappa-opioid receptor. FEBS Lett. **329:** 291–295.
15. NISHI, M., H. TAKESHIMA, K. FUKUDA, S. KATO & K. MORI. 1993. cDNA cloning and pharmacological characterization of an opioid receptor with high affinities for kappa-subtype-selective ligands. FEBS Lett. **330:** 77–80.
16. FUKUDA, K., S. KATO, K. MORI, M. NISHI & H. TAKESHIMA. 1993. Primary structures and expression from cDNAs of rat opioid receptor delta and mu-subtypes. FEBS Lett. **327:** 311–314.
17. MENG, F., G. X. XIE, R. C. THOMPSON, A. MANSOUR, A. GOLDSTEIN, S. J. WATSON & H. AKIL. 1993. Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc. Natl. Acad. Sci. USA **90:** 9954–9958.
18. THOMPSON, R. C., A. MANSOUR, H. AKIL & S. J. WATSON. 1993. Cloning and pharmacological characterization of a rat mu opioid receptor. Neuron **11:** 903–913.
19. BZDEGA, T., H. CHIN, H. KIM, H. H. JUNG, C. A. KOZAK & W. A. KLEE. 1993. Regional expression and chromosomal localization of the delta opiate receptor gene. Proc. Natl. Acad. Sci. USA **90:** 9305–9309.
20. GIOANNINI, T. L., A. D. HOWARD, J. M. HILLER & E. J. SIMON. 1985. Purification of an active opioid binding protein from bovine striatum. J. Biol. Chem. **260:** 15117–15121.
21. GIOANNINI, T. L., Y-H. YAO, J. M. HILLER, L. P. TAYLOR & E. J. SIMON. 1993. Antisera against peptides derived from a purified mu-opioid binding protein recognize the protein as well as mu-opioid receptors in brain regions and a cell line. Mol. Pharmacol. **44:** 796–801.
22. HILLER, J. M., Y. ZHANG, G. BING, T. L. GIOANNINI, E. A. STONE & E. J. SIMON. 1994. Immunohistochemical localization of mu opioid receptors in rat brain using antibodies generated against a peptide sequence present in a purified mu opioid binding protein. Neuroscience **62:** 829–841.
23. PEARSON, J., L. BRANDEIS, E. J. SIMON & J. M. HILLER. 1980. Radioautography of binding of tritiated diprenorphine to opiate receptors in the rat. Life Sci. **26:** 1047–1052.
24. WAMSLEY, J. K. 1983. Opioid receptors: Autoradiography. Pharmacol. Rev. **35:** 69–83.
25. MANSOUR, A. & S. J. WATSON. 1993. Anatomical distribution of opioid receptors in mammalians: An overview. *In* Opioids I. Handbook of Experimental Pharmacology. Vol. 104/I. A. Herz, H. Akil & E. J. Simon, Eds.: 79–105. Springer-Verlag. Heidelberg.
26. GIOANNINI, T. L., L. Q. FAN, L. HYDE, D. OFRI, Y-H. YAO, J. M. HILLER & E. J. SIMON. 1993. Reconstitution of a purified mu-opioid binding protein in liposomes: Selective, high affinity, GTP-γS-sensitive mu-opioid agonist binding is restored. Biochem. Biophys. Res. Commun. **194:** 901–908.